Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland. I.—*The Human Strain.*

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Introduction.

In order to gain a general idea of this important species of trypanosome, it will be necessary to study as many individual strains as possible. It may be thought unnecessary to describe each strain so much in detail, but without this it will be impossible to get any order out of the chaos which rules at present in the classification of the African species of trypanosomes pathogenic to man and the domestic animals.

Up to the present the Commission have only had an opportunity of working with five human strains. Four of these are from natives infected in the Sleeping-Sickness Area, Nyasaland, the fifth from an European who contracted the disease in Portuguese East Africa. It is intended, in later papers, to describe five strains from wild game and the same number from the tsetse fly, *Glossina morsitans.*

The human strains are named: I, Mkanyanga; II, E——; III, Chituluka; IV, Chipochola; and V, Chibibi.

I. *Morphology of Strain I, Mkanyanga.*

This has already been dealt with in a previous paper.*

II. *Morphology of Strain II, E——.*

The following table gives the average length of this trypanosome as found in goats, sheep, monkeys, dogs and rats, 1500 trypanosomes in all, and also the length of the longest and shortest:—

* 'Roy. Soc. Proc.,' 1912, B, vol. 85, p. 423.

Table I.—Measurements of the Length of the Trypanosome of Strain II, E——.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·2	36·0	15·0

The average length of the trypanosome of Strain II, in different species of animals, is as follows :—

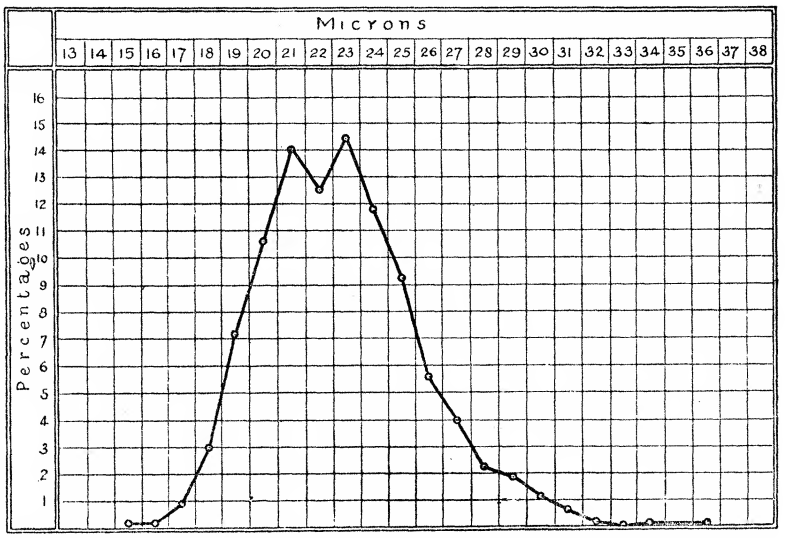
Table II.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	60	20·7	34·0	15·0
Sheep	20	21·3	28·0	18·0
Monkey	160	22·9	36·0	17·0
Dog	260	21·8	31·0	17·0
Rat	1000	23·1	32·0	17·0

Table III.—Distribution in respect to Length of 1500 Individuals of the Trypanosome of Strain II, E——.

	In microns.										
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	2	2	12	55	108	159	210	188	215	177	138
Percentage...	0·2	0·2	0·9	3·0	7·2	10·6	14·0	12·6	14·4	11·8	9·2
	In microns.										
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.
Total	83	60	34	26	18	8	3	—	1	—	1
Percentage...	5·6	4·0	2·3	1·8	1·2	0·6	0·2	—	0·1	—	0·1

CHART 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of Strain II, E——.



This curve is made up of measurements from 60 specimens of trypanosomes taken from the goat, 20 from the sheep, 160 from the monkey, 260 from the dog, and 1000 from the rat.

In a previous paper it was suggested that 1000 trypanosomes taken at random would be a suitable number to plot a curve from, for purposes of comparison. This is done in Chart 2.

The taking away of 500 rat trypanosomes has changed, to a great extent, the character of the curve. There is no resemblance between this curve and that given on Chart 1 of Strain I, Mkanyanga. If the two strains, I and II, belong to the same species, then little help can be expected from this system of measurement in classifying trypanosomes.

It has been suggested by Dr. J. W. W. Stephens that the measurements should be made from one animal, and he proposed the tame rat as a suitable species. There seems much to be said in favour of this. Practically, his proposal is that a series of slides should be made with blood taken on 10 consecutive days from a single rat, and that 100 trypanosomes should be drawn each day. But it is no light task to draw 1000 trypanosomes at a magnification of 2000, and afterwards to measure them. We have therefore made a compromise and measure 60 trypanosomes on nine consecutive days, beginning from the day the parasites first appear in the blood. In order to deal with a round number (500) only 20 are measured on the ninth day.

CHART 2.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain II, E——.

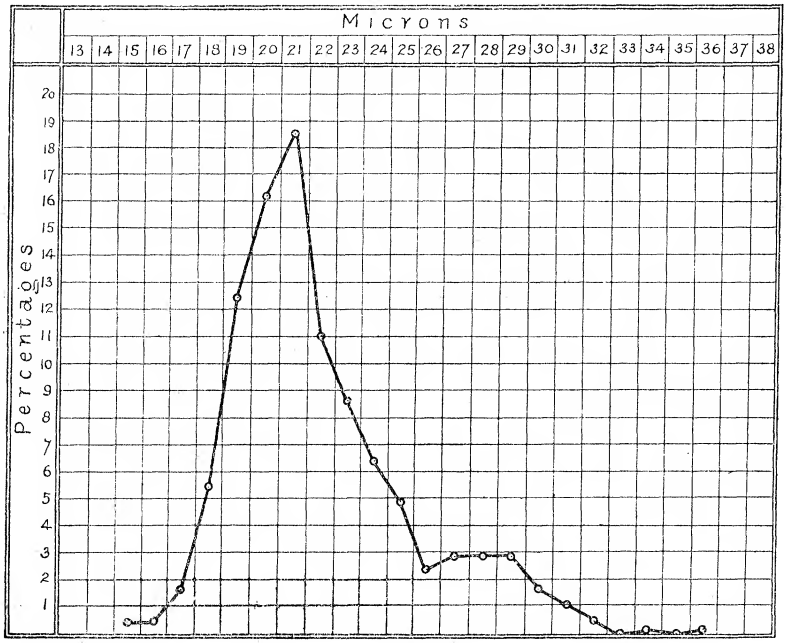
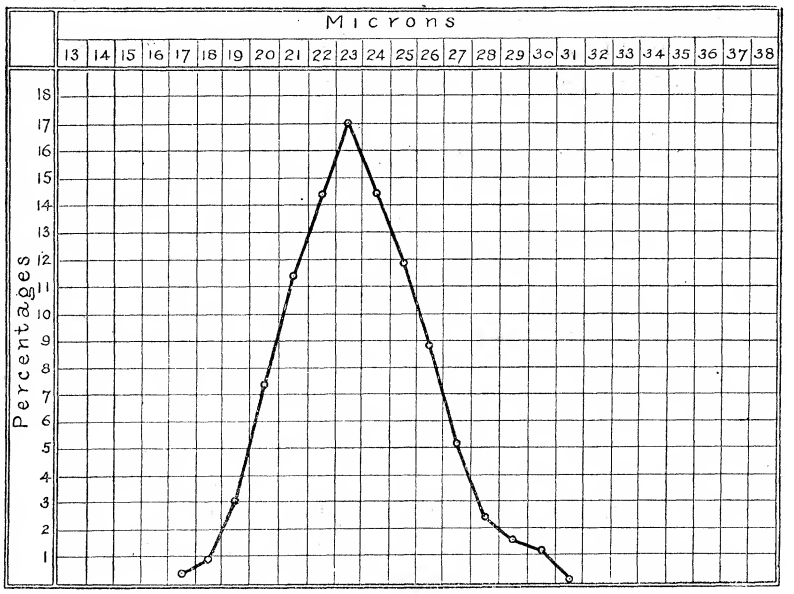


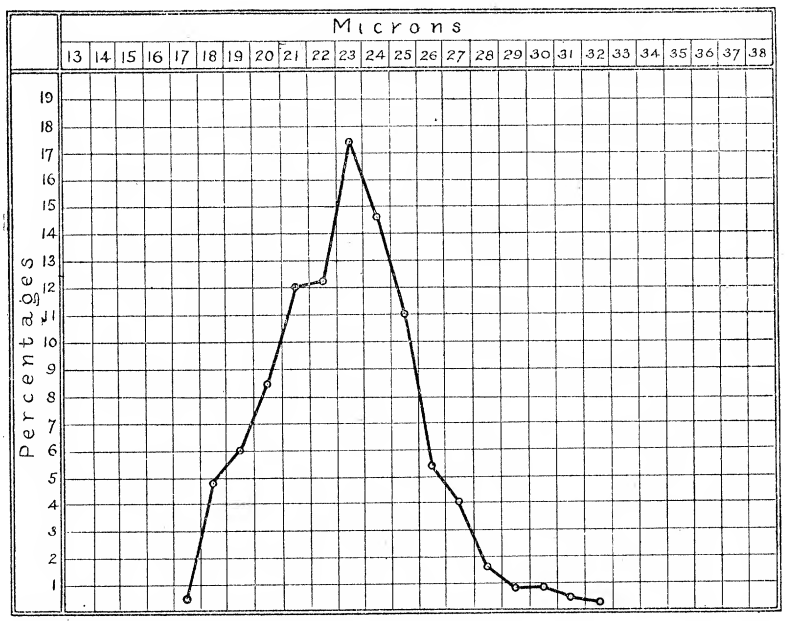
CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E——, taken on nine consecutive days from Rat 728.



This makes a symmetrical curve, which ascends and descends by fairly regular steps, but with little likeness to Charts 1 and 2.

In an organism low in the scale of nature, such as this, subject to great variation in form, it might be thought that it would not be likely to behave in any two rats in the same way. The following chart shows that this is not so, but that, on the contrary, the same strain of trypanosome planted in two different animals of the same species grows in a remarkably similar way.

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain II, E——, taken on nine consecutive days from Rat 726.



It is remarkable how much alike these last two curves are. If curves made in this way from different strains of one species of trypanosome showed the same degree of similarity, this method would certainly be useful for purposes of classification. But, as we have seen, the curve of Strain II has no resemblance to that of Strain I, and it will be found that each human strain of this species of trypanosome differs, more or less, when subjected to this method of measurement.

As the occurrence of posterior-nuclear forms has been made the distinguishing character between *Trypanosoma brucei, gambiense,* and *rhodesiense,* it will be of interest to note the percentage of these forms in the various strains. The method used is to count the number of posterior

nuclears in 1000 short and stumpy forms in 10 specimens of a single rat's blood taken, as near as possible, on 10 consecutive days.

Table IV.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain II, E——.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
June 25	728	Rat	10
„ 26	728	„	17
„ 27	728	„	3
„ 29	728	„	9
July 1	728	„	5
„ 2	728	„	5
„ 3	728	„	9
„ 4	728	„	3
„ 5	728	„	18
„ 6	728	„	14
		Average	9·3

In regard to breadth, shape, contents of cell, nucleus, micronucleus, undulating membrane and flagellum, it is not proposed to describe these characters separately for each strain, as was done in Strain I. Suffice it to say that no difference can be made out in regard to these particulars on comparing the five strains. The same posterior-nuclear and blunt-ended forms are present in all.

III. *Morphology of Strain III, Chituluka.*

The following table gives the average length of this trypanosome as found in the goat, monkey, dog and rat, 1500 trypanosomes in all, and also the length of the longest and shortest :—

Table V.—Measurements of the Length of the Trypanosome of Strain III, Chituluka.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	26·1	38·0	15·0

The average length of the trypanosome of Strain III, in different species of animals, is as follows :—

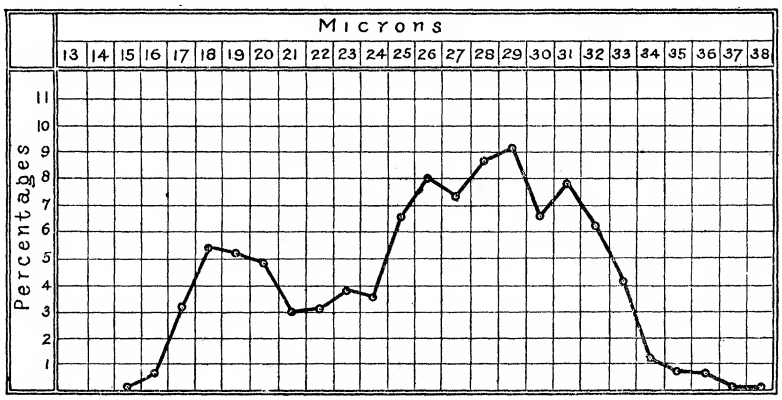
Table VI.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	26·9	32·0	16·0
Monkey	160	27·7	36·0	16·0
Dog	260	24·1	35·0	16·0
Rat	1000	26·4	38·0	15·0

Table VII.—Distribution in respect to Length of 1500 Individuals of the Trypanosome of Strain III, Chituluka.

	In microns.											
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.
Total	1	8	48	81	78	71	44	46	56	53	98	120
Percentages	0·1	0·6	3·2	5·4	5·2	4·8	3·0	3·1	3·8	3·6	6·6	8·0
	In microns.											
	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.
Total	111	128	138	99	117	91	63	27	11	9	1	1
Percentages	7·4	8·6	9·2	6·6	7·8	6·2	4·2	1·1	0·7	0·6	0·1	0·1

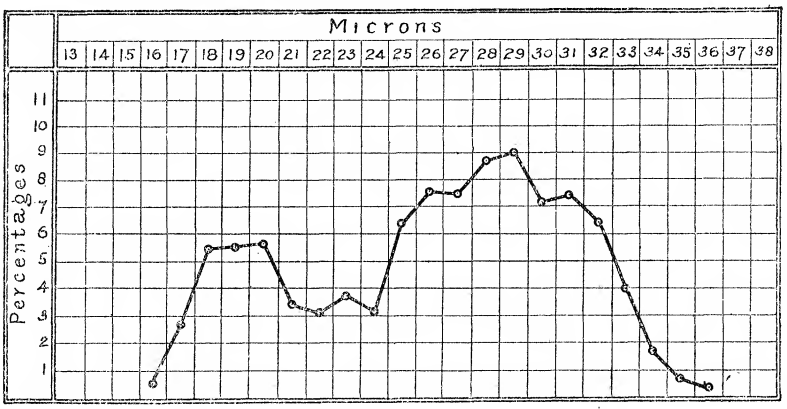
CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of Strain III, Chituluka.



This curve is made up of measurements from 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 1000 from the rat. It resembles that of Strain I, and differs absolutely from Strain II.

As in the case of Strain II, E——, a curve is also given of 1000 individuals of this strain.

CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain III, Chituluka.



This curve, made up of 1000 individuals, is very similar to the previous one of 1500. It is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

The two following curves represent measurements of 500 trypanosomes taken from each of two rats.

CHART 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 952.

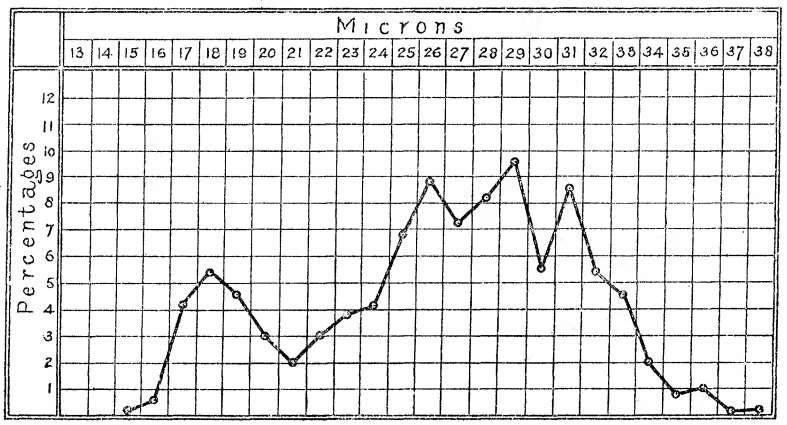
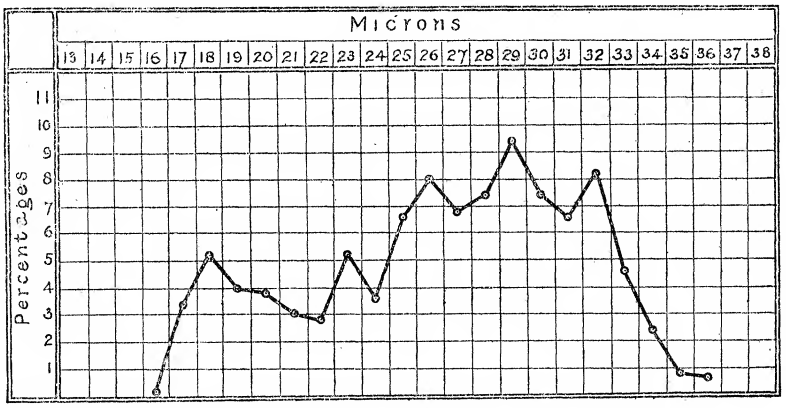


CHART 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain III, Chituluka, taken on nine consecutive days from Rat 953.



These last two curves from different rats also closely resemble each other. It is curious and striking that the same strain of trypanosome growing in two different animals should show this remarkable similarity.

Table VIII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain III, Chituluka.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
August 2	953	Rat	4
„ 3	953	„	6
„ 6	953	„	3
„ 7	953	„	8
„ 8	953	„	6
„ 9	953	„	13
„ 10	953	„	32
		Average	10·3

IV. *Morphology of Strain IV, Chipochola.*

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1000 trypanosomes in all, and also the length of the longest and shortest:—

Table IX.—Measurements of the Length of the Trypanosome of Strain IV, Chipochola.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·5	34·0	15·0

The average length of the trypanosome of Strain IV, in different species of animals, is as follows :—

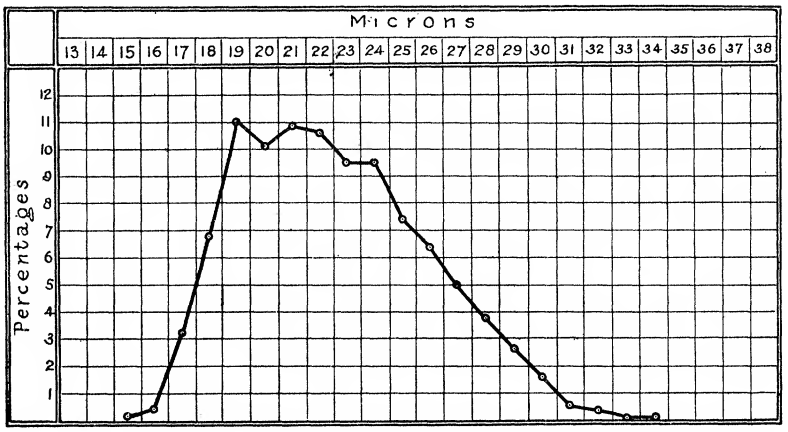
Table X.

Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	20·4	29·0	15·0
Monkey	160	22·0	34·0	16·0
Dog	260	20·9	31·0	15·0
Rat	500	22·5	34·0	15·0

Table XI.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of Strain IV, Chipochola.

	In microns.									
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.
Total	2	4	32	68	110	101	109	106	95	95
Percentages	0·2	0·4	3·2	6·8	11·0	10·1	10·9	10·6	9·5	9·5
	In microns.									
	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.
Total	74	64	50	38	26	16	5	3	1	1
Percentages	7·4	6·4	5·0	3·8	2·6	1·6	0·5	0·3	0·1	0·1

CHART 9.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain II, Chipochola.



This curve is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

CHART 10.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IV, Chipochola, taken on nine consecutive days from Rat 1337.

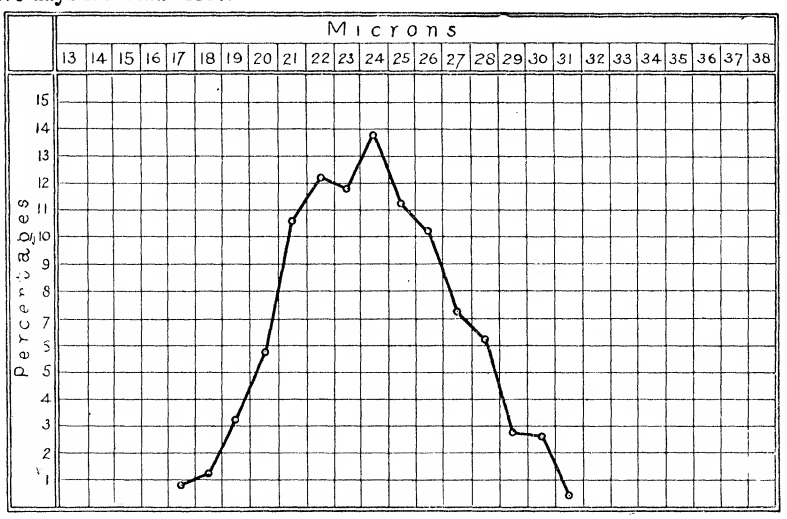


Table XII.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IV, Chipochola.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Sept. 20	1337	Rat	1
,, 23	1337	,,	1
,, 24	1337	,,	2
,, 25	1337	,,	4
,, 26	1337	,,	0
,, 28	1337	,,	0
,, 29	1337	,,	1
,, 30	1337	,,	14
Oct. 1	1337	,,	5
,, 2	1337	,,	5
		Average	3·3

V. *Morphology of Strain V, Chibibi.*

The following table gives the average length of this trypanosome as found in goats, monkeys, dogs and rats, 1000 in all, and also the length of the longest and shortest :—

Table XIII.—Measurements of the Length of the Trypanosome of Strain V, Chibibi.

Date.	Method of fixing.	Method of staining.	In microns.		
			Average length.	Maximum length.	Minimum length.
1912	Osmic acid	Giemsa	22·4	37·0	15·0

The average length of the trypanosome of Strain V, in different species of animals, is as follows :—

Table XIV.

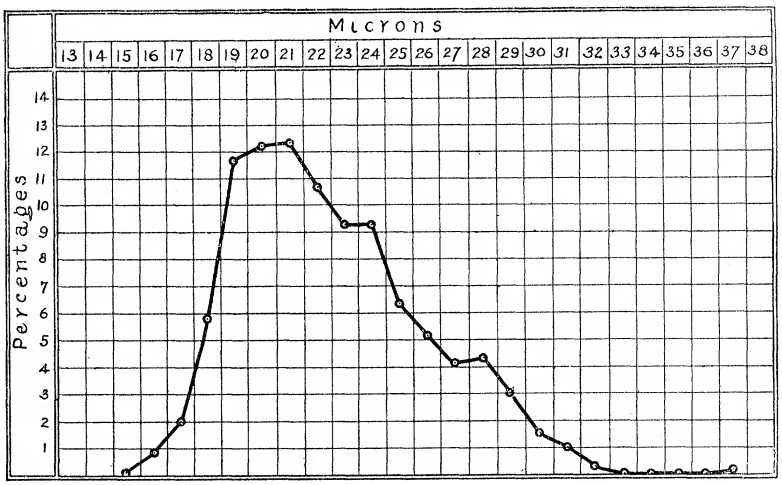
Species of animal.	Number of trypanosomes measured.	In microns.		
		Average length.	Maximum length.	Minimum length.
Goat	80	19·9	31·0	16·0
Monkey	160	21·8	32·0	15·0
Dog	260	20·6	37·0	16·0
Rat	500	24·0	32·0	18·0

Table XV.—Distribution in respect to Length of 1000 Individuals of the Trypanosome of Strain V, Chibibi.

	In microns.										
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Total	1	8	20	58	117	122	123	107	93	93	63
Percentages	0·1	0·8	2·0	5·8	11·7	12·2	12·3	10·7	9·3	9·3	6·3

	In microns.											
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.
Total	51	41	43	30	16	10	3	—	—	—	—	1
Percentages	5·1	4·1	4·3	3·0	1·6	1·0	0·3	—	—	—	—	0·1

CHART 11.—Curve representing the Distribution, by Percentages, in respect to Length, of 1000 Individuals of the Trypanosome of Strain V, Chibibi.



This curve is made up of 80 specimens of trypanosomes taken from the goat, 160 from the monkey, 260 from the dog, and 500 from the rat.

CHART 12.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain V, Chibibi, taken on nine consecutive days from Rat 1660.

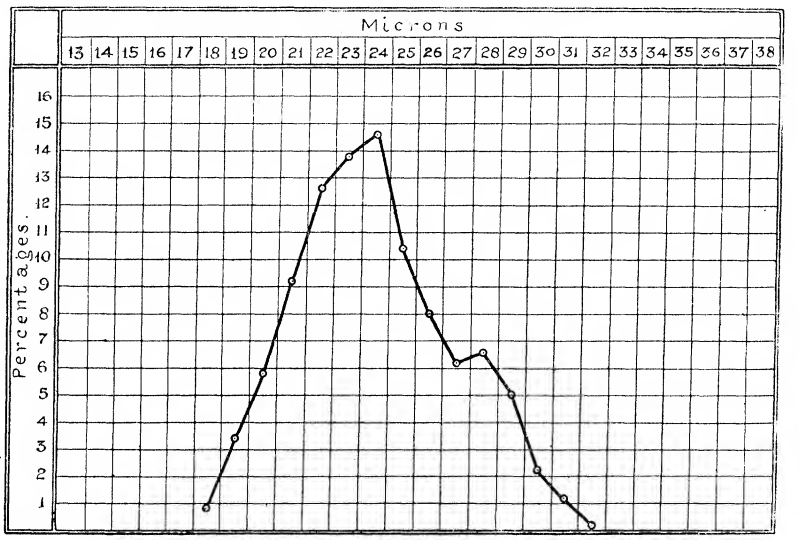


Table XVI.—Percentage of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain V, Chibibi.

Date.	Experiment No.	Animal.	Percentage among short and stumpy forms.
1912.			
Dec. 3	1660	Rat	0
,, 4	1660	,,	34
,, 5	1660	,,	2
,, 8	1660	,,	6
,, 9	1660	,,	8
,, 10	1660	,,	23
,, 11	1660	,,	31
,, 12	1660	,,	28
,, 13	1660	,,	27
,, 14	1660	,,	32
		Average	21·1

Comparison of the Human Strains.

The following table gives the average length of this trypanosome in the five human strains under consideration, as found in goats, sheep, monkeys, dogs and rats, 6200 trypanosomes in all, and also the length of the longest and shortest:—

Table XVII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from various animals.

Date.	Strain.	Name.	Number of trypano-somes.	Animals.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	1220	Various	24·1	36·0	14·0
1912	II	E——	1500	„	22·2	36·0	15·0
1912	III	Chituluka	1500	„	26·1	38·0	15·0
1912	IV	Chipochola	1000	„	22·5	34·0	15·0
1912	V	Chibibi	1000	„	22·4	37·0	15·0
			6220		23·5	38·0	14·0

It must be acknowledged that, in spite of the fairly large number of trypanosomes measured, there is a marked difference in the average length of the five human strains—from 22·2 to 26·1 microns. Strains II, IV and V are similar, varying only from 22·2 to 22·5.

This difference in average length may be due to slight variations having taken place in the different strains, resulting from the passage through more or less resistant man. There is no evidence that this variation is due to treatment by atoxyl or other drugs. It has been shown that the same strain grown in two animals of the same species gives like results.

Table XVIII.—Measurements of the Length of the Trypanosomes of the five Human Strains. The trypanosomes have been taken from the rat alone.

Date.	Strain.	Name.	Number of trypano-somes.	Animal.	In microns.		
					Average length.	Maximum length.	Minimum length.
1912	I	Mkanyanga	600	Rat	25·1	35·0	16·0
1912	II	E——	1000	„	23·1	32·0	17·0
1912	III	Chituluka	1000	„	26·4	38·0	15·0
1912	IV	Chipochola	500	„	22·5	34·0	15·0
1912	V	Chibibi	500	„	24·0	32·0	18·0
			3600		24·2	38·0	15·0

Comparison of the Curves from the Five Human Strains.

It must also be confessed that, on comparing the five curves one with another, they do not give as much assistance in classifying this species of trypanosome as was hoped. Curves I and III are alike, and coincide with that prepared by Dr. Stephens from the case of Armstrong in Liverpool, whereas Curves II, IV and V approach more to the type described by Kinghorn and Yorke from the Luangwa Valley.

Table XIX.—Distribution in respect to Length of 6220 Individuals of the five Human Strains. The trypanosomes have been taken at random from various animals.

	In microns.											
	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.
Strains I–V	1	10	41	154	325	494	528	577	512	525	511	464
Percentages	—	0·2	0·7	2·5	5·3	8·0	8·4	9·3	8·3	8·4	8·3	7·5

	In microns.											
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.
Strains I–V	425	372	347	307	198	167	123	77	36	12	11	2	1
Percentages	6·8	6·0	5·6	4·9	3·1	2·7	2·0	1·0	0·6	0·2	0·2	—	—

CHART 13.—Curve representing the Distribution, by Percentages, in respect to Length, of 6220 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland. The trypanosomes have been taken from various animals.

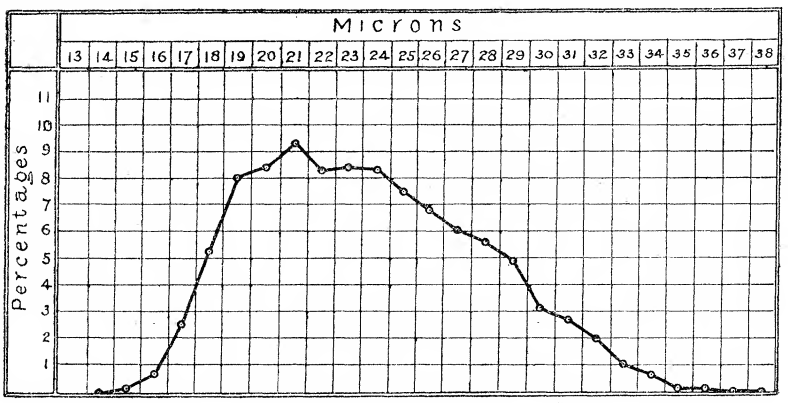
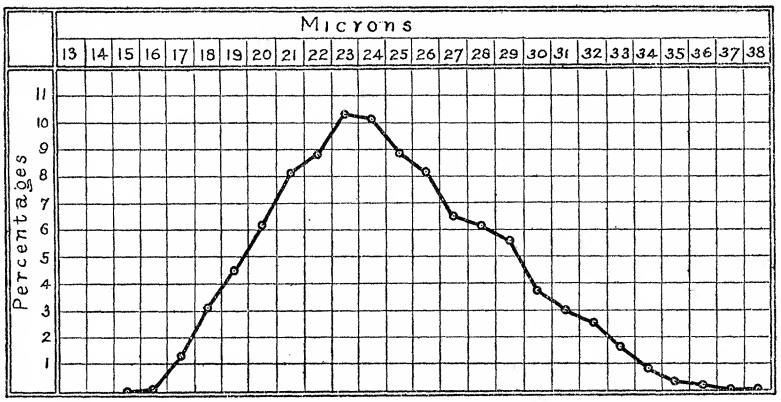


CHART 14.—Curve representing the Distribution, by Percentages, in respect to Length, of 3600 Individuals of the Human Strain of the Trypanosome causing Disease in Man in Nyasaland, taken from the rat alone.



Curves 13 and 14 will be found of use when the human strain of this species of trypanosome is compared with the Wild Game and the Wild *Glossina morsitans* strains.

Table XX.—Comparison of the Percentages of Posterior-Nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human Strain.

Experiment No.	Strain.	Name.	Animal.	Percentage among short and stumpy forms.
—	I	Mkanyanga	Rat	34·1
728	II	E——	,,	9·3
953	III	Chituluka	,,	10·3
1337	IV	Chipochola	,,	3·3
1660	V	Chibibi	,,	32·0
			Average	17·8

It is to be noted that in the human strain the percentage of posterior-nuclear forms varies greatly, although the method of enumeration is the same in each case. This presence of posterior-nuclear forms would have been accepted a few months ago as sufficient proof that the species dealt with was *T. rhodesiense*. Since then posterior-nuclear forms have been reported as occurring in *T. brucei* from Egypt, Uganda and Zululand. In a strain lately obtained by Theiler from the same spot in Zululand where this species was originally discovered in 1894, this percentage rose to the highest yet recorded.

Conclusions.

1. The five human strains of this trypanosome, isolated from four natives in Nyasaland and one European in Portuguese East Africa, belong to the same species.

2. This species is *T. rhodesiense* (Stephens and Fantham).

3. Evidence is accumulating that *T. rhodesiense* and *T. brucei* (Plimmer and Bradford) are identical.

Contributions to the Biochemistry of Growth.—The Glycogen-content of the Liver of Rats bearing Malignant New Growths.*

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In previous papers by one of us (1, 2) observations on the gaseous metabolism and on the nitrogen metabolism of tumour-bearing rats have been recorded. The present paper contains observations on the carbohydrate metabolism of tumour-bearing rats. The tumour-strain employed in our previous work was also used for these experiments; it is a spindle-celled sarcoma (J.R.S. of the Imperial Cancer Research Fund) which has a rapid growth. This tumour does not contain any glycogen. A large number of glycogen estimations were carried out with the liver of normal and of tumour-bearing animals. The glycogen estimations were made by Pflüger's method; the glucose obtained by the hydrolysis of glycogen was estimated in the first part of the work gravimetrically according to the technique used by Pflüger; later on, Bertrand's method of titration was employed. It is necessary to bear in mind that only 4 to 6 grm. of liver tissue are available for analysis in these estimations, so that with a glycogen percentage below 0·1 to 0·2 per cent. the absolute amount of glycogen present in the whole liver of a rat is so small that it cannot be determined

* This research is in continuation of papers in 'Roy. Soc. Proc.,' 1908, B, vol. 80, p. 263; 1910, vol. 82, p. 307; *ibid.*, p. 316.